

# Procaine

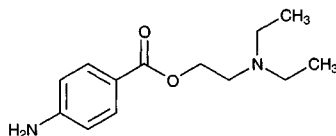
**Molecular formula:**  $C_{13}H_{20}N_2O_2$

**Molecular weight:** 236.31

**CAS Registry No.:** 59-46-1, 51-05-8 (HCl), 149-13-3 (borate)

**Merck Index:** 7937

**Lednicer No.:** 1 9



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## SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 100  $\mu$ L 1 M NaOH + 3 mL heptane:ethyl acetate 90:10, shake for 2 min, centrifuge at 1200 g for 10 min. Remove the organic phase and add it to 50  $\mu$ L 50 mM sulfuric acid, shake for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 820  $\mu$ g sodium acetate, inject a 40  $\mu$ L aliquot. (The sodium acetate was measured out by adding 50  $\mu$ L 200 mM sodium acetate in MeOH to the tube and evaporating the MeOH.)

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## HPLC VARIABLES

**Column:** 250  $\times$  4 10  $\mu$ m  $\mu$ Bondapak C18

**Mobile phase:** MeCN:10 mM  $\text{NaH}_2\text{PO}_4$  20:80, adjusted to pH 2.1

**Column temperature:** 30

**Flow rate:** 1

**Injection volume:** 40

**Detector:** UV 205

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## CHROMATOGRAM

**Retention time:** 10

**Limit of detection:** 5 ng/mL

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## OTHER SUBSTANCES

**Extracted:** lidocaine

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## KEY WORDS

plasma; rabbit

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## REFERENCE

Le Guévello,P.; Le Corre,P.; Chevanne,P.; Le Verge,R. High-performance liquid chromatographic determination of bupivacaine in plasma samples for biopharmaceutical studies and application to seven other local anaesthetics, *J.Chromatogr.*, **1993**, 622, 284–290.

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## SAMPLE

**Matrix:** blood

**Sample preparation:** Rock 5 mL whole blood + 10 mL water + 8.5 mL  $\text{Na}_2\text{WO}_4$  in a 50 mL stoppered tube for 1 min, add 6 mL  $\text{NiCl}_2$ , rock for 5 min, add 15 mL dichloromethane:isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90  $\mu$ m filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500  $\mu$ L MeCN: water 80:20, inject a 20  $\mu$ L aliquot. ( $\text{Na}_2\text{WO}_4$  prepared by mixing 10 g  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$  in 38 mL of 2 M NaOH and 2.5 g of  $\text{NaHCO}_3$  and making up to 100 mL.  $\text{NiCl}_2$  was 17% w/v  $\text{NiCl}_2$  in water.)

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## HPLC VARIABLES

**Column:** 200  $\times$  4.6 5  $\mu$ m Hypersil C8

**Mobile phase:** Gradient A = MeCN; B = 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min

**Injection volume:** 20

**Detector:** UV 230

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**CHROMATOGRAM****Retention time:** 11**Limit of detection:** 0.20 ppm

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**OTHER SUBSTANCES****Extracted:** buprenorphine, caffeine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, papaverine, pentazocine**Also analyzed:** bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam, oxazepam

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**KEY WORDS**whole blood

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**REFERENCE**Bernal, J.L.; Del Nozal, M.J.; Rosas, V.; Villarino, A. Extraction of basic drugs from whole blood and determination by high performance liquid chromatography, *Chromatographia*, **1994**, 38, 617–623.

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**SAMPLE****Matrix:** blood**Sample preparation:** 100  $\mu$ L Plasma + 100  $\mu$ L 20  $\mu$ g/mL caffeine + 8 mL dichloromethane, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200  $\mu$ L mobile phase, inject a 20  $\mu$ L aliquot.

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**HPLC VARIABLES****Column:** 150  $\times$  6 Shimpack CLS-ODS (Shimadzu)**Mobile phase:** MeCN:MeOH:0.5 mM phosphoric acid 6:2.5:91.5**Column temperature:** 40**Flow rate:** 1.5**Injection volume:** 20**Detector:** UV 290

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**CHROMATOGRAM****Internal standard:** caffeine

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**KEY WORDS**plasma; rat

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**REFERENCE**Lee, C.K.; Uchida, T.; Kitagawa, K.; Yagi, A.; Kim, N.-S.; Goto, S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, 83, 562–565.

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**SAMPLE****Matrix:** blood, urine**Sample preparation:** 200  $\mu$ L Serum or urine + 100  $\mu$ L 0.072  $\mu$ g/mL procaine hydrochloride (+ 100  $\mu$ L 6.25% NaHCO<sub>3</sub> solution for urine samples) + 5 mL dichloromethane, vortex 210 s. Evaporate organic phase to dryness under nitrogen, take up in 100  $\mu$ L mobile phase, inject 20  $\mu$ L.

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**HPLC VARIABLES****Column:** 250  $\times$  4.6 Partisil-10 ODS-3**Mobile phase:** MeCN:10 mM pH 4.8 potassium phosphate buffer 7:93**Flow rate:** 2**Injection volume:** 20**Detector:** UV 228

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**CHROMATOGRAM****Retention time:** 12.9**Internal standard:** procaine**Limit of detection:** 100 ng/mL

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**OTHER SUBSTANCES**

**Extracted:** cimetidine

**Noninterfering:** cimetidine sulfoxide, caffeine, minoxidil, furosemide, propranolol, sulfinpyrazone, flurazepam, diazepam, methyl dopa, acetaminophen, digoxin, quinidine

**Interfering:** procainamide, tolazamide

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**KEY WORDS**

serum; procaine is IS

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**REFERENCE**

Guay,D.R.; Bockbrader,H.N.; Matzke,G.R. High-performance liquid chromatographic analysis of cimetidine in serum and urine, *J.Chromatogr.*, **1982**, 228, 398–403.

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**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Urine. Adjust pH of urine to 5 before freezing. 5 mL Urine + 3-30  $\mu$ g tetracaine, adjust pH to 9.5 with borax buffer, extract twice with 7 mL portions of dichloromethane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL MeOH, inject a 20-100  $\mu$ L aliquot. Blood. 4 mL Plasma or whole blood + 1  $\mu$ g tetracaine, adjust pH to 9.5 with borax buffer, extract twice with 7 mL portions of dichloromethane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 0.2-2 mL MeOH, inject a 125  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 10  $\mu$ m Radial Pak C18

**Mobile phase:** MeCN:16.5 mM triethylamine 85:15, pH adjusted to 3 with concentrated phosphoric acid

**Flow rate:** 2

**Injection volume:** 20-125

**Detector:** UV 288

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**CHROMATOGRAM**

**Internal standard:** tetracaine

**Limit of detection:** 10 ng/mL (urine), 1 ng/mL (plasma)

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**KEY WORDS**

horse; plasma; whole blood; pharmacokinetics

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**REFERENCE**

Stevenson,A.J.; Weber,M.P.; Todt,F.; Mendonca,M.; Fenwick,J.D.; Young,L.; Kwong,E.; Chen,F.; Beaumier,P.; Timmings,S.; Woodard,W.; Kacew,S. Determination of procaine in equine plasma and urine by high-performance liquid chromatography, *J.Anal.Toxicol.*, **1992**, 16, 93–96.

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**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** 2 mL Whole blood, plasma, or urine + 1 mL saturated sodium carbonate + 50  $\mu$ L 10  $\mu$ g/mL butacaine, add to a 3 mL Extrelut SPE cartridge, elute with 15 mL dichloromethane. Evaporate eluate to dryness under a stream of nitrogen at 40°, reconstitute in 100  $\mu$ L mobile phase, inject a 40  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Guard column:** 5  $\times$  6  $\mu$ Bondapak Guard Pak

**Column:** 300  $\times$  3.9 10  $\mu$ m  $\mu$ Bondapak C18

**Mobile phase:** MeCN:100 mM ammonium acetate 50:50

**Flow rate:** 1.5

**Injection volume:** 40

**Detector:** UV 280

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**CHROMATOGRAM**

**Retention time:** 4

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**Internal standard:** butacaine (10)

**Limit of detection:** 20 ng/mL

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#### OTHER SUBSTANCES

**Extracted:** tetracaine, p-aminobenzoic acid

**Also analyzed:** articaine, prilocaine, o-toluidine, lidocaine, bupivacaine, etidocaine, dibucaine, caffeine, amphetamine, ephedrine, epinephrine, morphine, diamorphine, ethylmorphine, acetylcodeine

**Interfering:** codeine, monoacetylmorphine

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#### KEY WORDS

whole blood; plasma; SPE

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#### REFERENCE

Rop,P.P.; Grimaldi,F.; Bresson,M.; Fornaris,M.; Viala,A. Liquid chromatographic analysis of cocaine, benzoyl-ecgonine, local anaesthetic agents and some of their metabolites in biological fluids, *J.Liq.Chromatogr.*, **1993**, 16, 2797-2811.

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#### SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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#### HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18

**Column:** 250 × 4.6 5 µm Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 292.6

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#### CHROMATOGRAM

**Retention time:** 5.218

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#### KEY WORDS

whole blood

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#### REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, 763, 149-163.

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#### SAMPLE

**Matrix:** bulk

**Sample preparation:** Prepare a 750 µg/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 µm), inject a 15 µL aliquot.

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#### HPLC VARIABLES

**Guard column:** 4 × 4 5 µm LiChrospher 100

**Column:** 125 × 4 3 µm Spherisorb ODS-1

**Mobile phase:** Gradient. A was water containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. B was MeCN:water 90:10 containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. A:B from 91:9 to 86:14 over 4 min, maintain at 86:14 for 13 min, to 55:45 over 11 min, maintain at 55:45 for 8 min, re-equilibrate at initial conditions for 20 min.

**Flow rate:** 0.7

**Injection volume:** 15

**Detector:** UV 210

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#### CHROMATOGRAM

**Retention time:** 4.3

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#### OTHER SUBSTANCES

**Simultaneous:** acetaminophen, acetylcodeine, benzocaine, caffeine, cocaine, codeine, diamorphine, lidocaine, 6-monoacetylmorphine, morphine, noscapine, papaverine

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#### REFERENCE

Grogg-Sulser,K.; Helmlin,H.-J.; Clerc,J.-T. Qualitative and quantitative determination of illicit heroin street samples by reversed-phase high-performance liquid chromatography: method development by CARTAGO-S, *J.Chromatogr.A*, **1995**, 692, 121-129.

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#### SAMPLE

**Matrix:** formulations

**Sample preparation:** Grind tablets, add 3-20 mL MeCN:water 15:85, sonicate for 10 min, filter, make up to 100 mL with MeCN:water 15:85. Remove a 500  $\mu$ L aliquot and add it to 300  $\mu$ L 250  $\mu$ g/mL procaine hydrochloride in water, make up to 10 mL with mobile phase, inject a 50  $\mu$ L aliquot.

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#### HPLC VARIABLES

**Column:** 250  $\times$  4.6 5  $\mu$ m ASI chromosphere 3869 octadecylsilane (Analytical Sciences, Inc.)

**Mobile phase:** MeCN:50 mM NaH<sub>2</sub>PO<sub>4</sub> 30:70 containing sodium pentanesulfonate, pH adjusted to 2.5 with concentrated phosphoric acid

**Flow rate:** 1

**Injection volume:** 50

**Detector:** E, Metrohm model E-611, Bioanalytical Systems Kel F cell, glassy carbon electrode + 1300 mV, auxiliary platinum electrode, Ag/AgCl reference electrode

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#### CHROMATOGRAM

**Retention time:** 5.7

**Internal standard:** procaine

**Limit of quantitation:** 1250 ng/mL

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#### OTHER SUBSTANCES

**Simultaneous:** guanethidine, hydrochlorothiazide

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#### KEY WORDS

tablets; not stability-indicating; procaine is IS

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#### REFERENCE

Stewart,J.T.; Clark,S.S. Liquid chromatographic determination of guanethidine salts and hydrochlorothiazide using electrochemical detection and ion-pair techniques, *J.Pharm.Sci.*, **1986**, 75, 413-415.

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#### SAMPLE

**Matrix:** formulations

**Sample preparation:** Make up 1 mL syrup to 50 mL with water. Remove a 2 mL aliquot and add it to 2 mL 0.1% procaine hydrochloride in water, make up to 100 mL with water, filter (0.45  $\mu$ m), inject a 50  $\mu$ L aliquot.

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#### HPLC VARIABLES

**Column:** 250  $\times$  4.6 Partisil ODS-3 C18

**Mobile phase:** MeCN:buffer:triethanolamine:water 15:80:0.2:4.8, pH adjusted to 4.5 with glacial acetic acid (Buffer was 4.72 g sodium acetate and 1.8 mL acetic acid in 1 L water.)

**Flow rate:** 1.2  
**Injection volume:** 50  
**Detector:** UV 254

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**CHROMATOGRAM**  
**Retention time:** 7.5  
**Internal standard:** procaine

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**OTHER SUBSTANCES**  
**Simultaneous:** procainamide

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**KEY WORDS**  
syrup; procaine is IS

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**REFERENCE**  
Alexander,K.S.; Pudipeddi,M.; Parker,G.A. Stability of procainamide hydrochloride syrups compounded from capsules, *Am.J.Hosp.Pharm.*, **1993**, 50, 693–698.

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**SAMPLE**  
**Matrix:** perfusate  
**Sample preparation:** Adjust pH of 5-10 mL perfusate to 5 with 180  $\mu$ L 2.5 M HCl, extract twice with an equal volume of ethyl acetate. Combine the organic layers, add 1 mL water, evaporate them to 1 mL under vacuum, inject a 20  $\mu$ L aliquot.

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**HPLC VARIABLES**  
**Column:** 300  $\times$  3.9 10  $\mu$ m  $\mu$ Bondapak C18  
**Mobile phase:** MeOH:water containing 30  $\mu$ L/L triethylamine, adjusted to pH 2.3 with phosphoric acid 10:90  
**Flow rate:** 1.5  
**Injection volume:** 20  
**Detector:** UV 290

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**CHROMATOGRAM**  
**Retention time:** 8.2

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**OTHER SUBSTANCES**  
**Extracted:** p-aminohippuric acid, aminobenzoic acid, 4-acetamidobenzoic acid

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**KEY WORDS**  
rabbit; chinchilla; pharmacokinetics

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**REFERENCE**  
Henrikus,B.M.; Kampffmeyer,H.G. Ester hydrolysis and conjugation reactions in intact skin and skin homogenate, and by liver esterase of rabbits, *Xenobiotica*, **1992**, 22, 1357–1366.

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**SAMPLE**  
**Matrix:** solutions

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**HPLC VARIABLES**  
**Guard column:** 4  $\times$  4 5  $\mu$ m LiChrospher100RP-18  
**Column:** 125  $\times$  4 5  $\mu$ m Spherisorb ODS 2  
**Mobile phase:** Gradient. MeOH:buffer from 10:90 to 30:70 over 5 min, to 10:90 over 2 min. (Buffer was 20 mM sodium acetate containing 0.28% triethylamine, adjusted to pH 4.5 with acetic acid.)  
**Flow rate:** 1.5  
**Detector:** UV 260

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**CHROMATOGRAM**  
**Retention time:** k' 3.5  
**Internal standard:** caffeine

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**OTHER SUBSTANCES**

**Simultaneous:** 4-aminobenzoic acid

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**REFERENCE**

Yang,H.; Thyron,F.C. Determination of six pharmaceuticals and their degradation products in reversed-phase high performance liquid chromatography by using amine additives, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1347-1357.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Guard column:** 45 × 4.6 5 µm Ultrasphere ODS

**Column:** 150 × 4.6 5 µm Ultrasphere ODS

**Mobile phase:** MeCN:MeOH:2.5 mM hexanesulfonic acid 35:40:25, adjusted to pH 6.0 with 100 mM acetic acid

**Flow rate:** 2

**Detector:** UV 310

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**CHROMATOGRAM**

**Retention time:** 2.6

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**OTHER SUBSTANCES**

**Simultaneous:** tetracaine

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**REFERENCE**

Asavapichayont,P.; Hu,J.; Foldvari,M. Development of an HPLC method for simultaneous analysis of tetracaine and its metabolite in dosage forms and biological fluids, with comparison to capillary electrophoresis method (Abstract 3307), *Pharm.Res.*, **1997**, *14*, S565.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

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**HPLC VARIABLES**

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

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**CHROMATOGRAM**

**Retention time:** 2.7

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**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclozine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl,

isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylegonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocanide, tolpropamine, tolycaine, tranlylcypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

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## REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, 323, 191–225.

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## SAMPLE

**Matrix:** solutions

**Sample preparation:** Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.

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## HPLC VARIABLES

**Column:** 300 × 3.9 10 µm µBondapak C18

**Mobile phase:** MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78

**Flow rate:** 1.5

**Injection volume:** 10

**Detector:** UV

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## CHROMATOGRAM

**Retention time:** k' 1.15

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## REFERENCE

Roos, R.W.; Lau-Cam, C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, 370, 403–418.

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## SAMPLE

**Matrix:** solutions

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## HPLC VARIABLES

**Column:** 150 × 4.6 Supelcosil LC-ABZ

**Mobile phase:** MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65

**Flow rate:** 1.5

**Injection volume:** 25

**Detector:** UV 254

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## CHROMATOGRAM

**Retention time:** 2.241



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**OTHER SUBSTANCES**

**Also analyzed:** 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methypylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, terfenadine

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**REFERENCE**

Asch, T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, 12(3), 18–21.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Guard column:** 30 × 3.2 7 µm SI 100 ODS (not commercially available)

**Column:** 150 × 3.2 7 µm SI 100 ODS (not commercially available)

**Mobile phase:** MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH<sub>2</sub>PO<sub>4</sub> and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)

**Flow rate:** 0.5–1

**Detector:** UV 216, 290

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**CHROMATOGRAM**

**Retention time:** 1.5

**Internal standard:** 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

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**OTHER SUBSTANCES**

**Also analyzed:** aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

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**REFERENCE**

Below, E.; Burrmann, M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J. Liq. Chromatogr.*, **1994**, 17, 4131–4144.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4.5 µm LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

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**CHROMATOGRAM**

**Retention time:** 7.25 (A), 4.03 (B)

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**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flur-

azepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mafenidol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, piroxicam, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfapyrazole, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocinide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

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**KEY WORDS**

details of plasma extraction

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**REFERENCE**

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J. Chromatogr. A*, **1995**, 692, 103–119.

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# Procarbazine

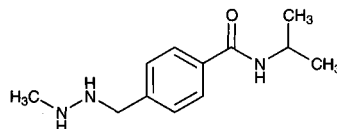
**Molecular formula:** C<sub>12</sub>H<sub>19</sub>N<sub>3</sub>O

**Molecular weight:** 221.30

**CAS Registry No.:** 671-16-9, 366-70-1 (HCl)

**Merck Index:** 7938

**Lednicer No.:** 2 27



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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

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**HPLC VARIABLES**

**Column:** 300 × 3.9 µm NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH<sub>2</sub>PO<sub>4</sub> adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

**Column temperature:** 30

**Flow rate:** 0.8

**Injection volume:** 50

**Detector:** UV 231

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**CHROMATOGRAM**

**Retention time:** 3.69

**Limit of detection:** <120 ng/mL

**KEY WORDS**

whole blood; plasma; interferences may occur—compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; tolaxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vandesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phenacyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thiopropazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

**REFERENCE**

Tracqui, A.; Kintz, P.; Mangin, P. Systematic toxicological analysis using HPLC/DAD, *J. Forensic Sci.*, **1995**, *40*, 254–262.

**SAMPLE**

**Matrix:** blood, microsomal incubation

**Sample preparation:** Rat plasma, microsomal incubation. 1 mL Rat plasma or microsomal incubation, extract with five 3 mL portions of cold diethyl ether. Evaporate combined ether extracts to dryness under a stream of nitrogen. Redissolve the residue in 250–500  $\mu$ L MeOH, add 10  $\mu$ L 670  $\mu$ g/mL IS in MeOH. Vortex, centrifuge at 12000 g for 1 min, inject a 15  $\mu$ L aliquot. Human plasma. 1 mL Human plasma, extract with three 4 mL portions of diethyl ether. Evaporate combined ether extracts to dryness under a stream of nitrogen. Re-dissolve the residue in 20  $\mu$ L MeOH, add 5  $\mu$ L 670  $\mu$ g/mL IS. Centrifuge at 12000 g for 1 min, inject a 15–20  $\mu$ L aliquot.

**HPLC VARIABLES**

**Column:** 300  $\times$  3.9 10  $\mu$ m  $\mu$ Bondapak C18

**Mobile phase:** MeOH:water 80:20

**Flow rate:** 2.0

**Injection volume:** 15–20

**Detector:** UV 254

**CHROMATOGRAM**

**Internal standard:** 4-methylacetophenone

**Limit of detection:** 2 nmol/mL

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**OTHER SUBSTANCES**

**Extracted:** metabolites

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**KEY WORDS**

human; plasma; rat; liver; pharmacokinetics

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**REFERENCE**

Shiba,D.A.; Weinkam,R.J. Quantitative analysis of procarbazine, procarbazine metabolites and chemical degradation products with application to pharmacokinetic studies, *J.Chromatogr.*, **1982**, 229, 397–407.

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**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Filter (0.45  $\mu\text{m}$ ) plasma or urine, inject an aliquot.

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**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 Partisil PXS 10/25 PAC

**Mobile phase:** MeOH:100 mM pH 7  $(\text{NH}_4)_2\text{H}_2\text{PO}_4$  10:90

**Flow rate:** 0.4

**Injection volume:** 10

**Detector:** E, Bioanalytical systems model TL-3, carbon paste working electrode +0.75 V, Ag/AgCl reference electrode, resurface electrode daily (glassy carbon electrode may be superior)

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**CHROMATOGRAM**

**Retention time:** 14

**Limit of detection:** 2 ng

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**KEY WORDS**

protect from light; plasma

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**REFERENCE**

Rucki,R.J.; Ross,A.; Moros,S.A. Application of an electrochemical detector to the determination of procarbazine hydrochloride by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, 190, 359–365.

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**SAMPLE**

**Matrix:** reaction mixtures

**Sample preparation:** Centrifuge, inject a 20  $\mu\text{L}$  aliquot.

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**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 5  $\mu\text{m}$  Microsorb C8

**Mobile phase:** MeOH:0.4 g/L  $(\text{NH}_4)_2\text{H}_2\text{PO}_4$  + 0.1% triethylamine (pH 10.0) 50:50

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 5.4

**Limit of detection:** 32000 ng/mL

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**REFERENCE**

Lunn,G.; Sansone,E.B. Reductive destruction of dacarbazine, procarbazine hydrochloride, isoniazid, and iproniazid, *Am.J.Hosp.Pharm.*, **1987**, 44, 2519–2524.

# Prochlorperazine

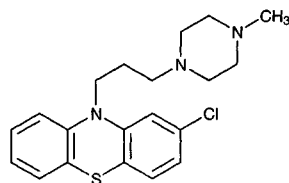
**Molecular formula:**  $C_{20}H_{24}ClN_3S$

**Molecular weight:** 373.95

**CAS Registry No.:** 58-38-8, 84-02-6 (maleate), 1257-78-9 (edisylate)

**Merck Index:** 7942

**Lednicer No.:** 1 381



## SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Plasma + 1 mL water + 500  $\mu$ L saturated sodium carbonate solution, vortex for 5 s, add 5 mL pentane:isopropanol 97:3, shake for 15 min, centrifuge at 1725 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness at 65°, reconstitute the residue in 200  $\mu$ L MeCN, inject a 100  $\mu$ L aliquot.

## HPLC VARIABLES

**Column:** 250  $\times$  4.6 5  $\mu$ m Zorbax CN

**Mobile phase:** MeCN:100 mM ammonium acetate buffer 90:10

**Flow rate:** 4

**Injection volume:** 100

**Detector:** E, Bioanalytical Systems, +0.9 V

## CHROMATOGRAM

**Retention time:** 5.56

**Internal standard:** prochlorperazine

## OTHER SUBSTANCES

**Extracted:** trimeprazine

## KEY WORDS

plasma; prochlorperazine is IS

## REFERENCE

McKay,G.; Cooper,J.K.; Midha,K.K.; Hall,K.; Hawes,E.M. Simple and sensitive high-performance liquid chromatographic procedure with electrochemical detection for the determination of plasma concentrations of trimeprazine following single oral doses, *J.Chromatogr.*, **1982**, 233, 417-422.

## SAMPLE

**Matrix:** blood

**Sample preparation:** 2-5 mL Plasma + 10-25 ng methotrimeprazine + 1 mL 1 M NaOH + 8 mL diethyl ether:chloroform 4:1, shake for 10 min, centrifuge. Remove the upper organic layer and evaporate it to dryness at 50° under a stream of nitrogen. Dissolve the residue in 50  $\mu$ L mobile phase, inject an aliquot.

## HPLC VARIABLES

**Column:** 150  $\times$  4.6 5  $\mu$ m Spherisorb nitrile-bonded silica

**Mobile phase:** MeCN:MeOH:100 mM  $K_2HPO_4$  adjusted to pH 6.5 with orthophosphoric acid 6:4:7

**Detector:** E, Model LCA 15-EDT Research, glassy carbon electrode +0.85 V

## CHROMATOGRAM

**Retention time:** 6

**Internal standard:** methotrimeprazine (4)

**Limit of detection:** 0.2 ng/mL

## OTHER SUBSTANCES

**Noninterfering:** chlorpromazine

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**KEY WORDS**plasma

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**REFERENCE**

Sankey,M.G.; Holt,J.E.; Kaye,C.M. A simple and sensitive H.P.L.C. method for the assay of prochlorperazine in plasma, *Br.J.Clin.Pharmacol.*, **1982**, 13, 578–580.

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**SAMPLE****Matrix:** blood

**Sample preparation:** 2 mL Plasma + 1 mL water, vortex for 10 s, add 500  $\mu$ L saturated sodium carbonate, vortex, add 5 mL pentane:isopropanol 97:3, mix for 20 min, centrifuge at 1725 g for 5 min, remove the organic layer and repeat the extraction. Combine the organic layers and evaporate them to dryness at 65° after adding a few anti-bumping granules. Cool, add 200  $\mu$ L MeCN, mix for 20 s, inject a 100  $\mu$ L aliquot.

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**HPLC VARIABLES****Column:** 250  $\times$  4.6 10  $\mu$ m Spherisorb CN**Mobile phase:** MeCN:100 mM ammonium acetate 90:10**Flow rate:** 4**Injection volume:** 100**Detector:** E, Bioanalytical Systems Model LC4A, glassy carbon electrode +0.9 V, fixed 10 nA feed

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**CHROMATOGRAM****Retention time:** 4.4**Internal standard:** prochlorperazine

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**OTHER SUBSTANCES****Simultaneous:** chlorpromazine

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**KEY WORDS**plasma; prochlorperazine is IS

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**REFERENCE**

Cooper,J.K.; McKay,G.; Midha,K.K. Subnanogram quantitation of chlorpromazine in plasma by high-performance liquid chromatography with electrochemical detection, *J.Pharm.Sci.*, **1983**, 72, 1259–1262.

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**SAMPLE****Matrix:** blood

**Sample preparation:** 2 mL Plasma + 30 ng chlorpromazine in MeOH + 200  $\mu$ L 5 M NaOH + 10 mL chloroform, shake for 10 min, stand in an ice bath for at least 30 min, centrifuge at 4° at 2800 rpm (RCF = 1578) for 10 min. Remove the organic layer and evaporate it under nitrogen at 40°. Dissolve the residue in 200  $\mu$ L mobile phase, inject.

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**HPLC VARIABLES****Column:** 250  $\times$  4.6 5  $\mu$ m Spherisorb nitrile**Mobile phase:** MeCN:100 mM (NH<sub>4</sub>)H<sub>2</sub>PO<sub>4</sub> + 50 mg/L EDTA adjusted to pH 6.5 with ammonia 60:40**Flow rate:** 2**Injection volume:** 200**Detector:** E, Bioanalytical Systems LC-4B, glassy carbon electrode 0.85 V

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**CHROMATOGRAM****Retention time:** 6**Internal standard:** chlorpromazine (4)**Limit of quantitation:** 1 ng/mL

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**KEY WORDS**

plasma

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**REFERENCE**

Fowler,A.; Taylor,W.; Bateman,D.N. Plasma prochlorperazine assay by high-performance liquid chromatography-electrochemistry, *J.Chromatogr.*, **1986**, 380, 202-205.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Adjust pH of 1 mL plasma to 9.0 with 1 M NaOH, add 6 mL n-hexane:ethyl acetate 1:2, shake for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and add it to 100  $\mu$ L pH 2.4  $\text{KH}_2\text{PO}_4$ , shake for 30 s, centrifuge at 3000 g for 3 min, inject a 50  $\mu$ L aliquot of the organic layer.

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**HPLC VARIABLES**

**Column:** 120  $\times$  4.6 5  $\mu$ m Nucleosil C18

**Mobile phase:** MeCN:MeOH:buffer 26:4:70 (Buffer was 10 mM  $\text{KH}_2\text{PO}_4$  containing 5 mM tetramethylammonium chloride adjusted to pH 2.4 with 85% phosphoric acid.)

**Flow rate:** 1

**Injection volume:** 50

**Detector:** E, ESA Coulochem Model 5100A, detector 1 +0.20 V, detector 2 +0.73 V, guard cell 0.75 V

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**CHROMATOGRAM**

**Retention time:** 17.80

**Internal standard:** prochlorperazine

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**OTHER SUBSTANCES**

**Extracted:** perphenazine

**Simultaneous:** alprazolam, chlorpheniramine, diltiazem, lorazepam, mesoridazine, nifedipine, ranitidine

**Noninterfering:** doxepin, metoprolol, nordoxepin, nortriptyline, propranolol, theophylline, tri-fluoperazine, trihexyphenidyl, verapamil

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**KEY WORDS**

plasma; protect from light; prochlorperazine is IS

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**REFERENCE**

Foglia,J.P.; Sorisio,D.; Kirshner,M.A.; Mulsant,B.H.; Perel,J.M. Quantitative determination of perphenazine and its metabolites in plasma by high-performance liquid chromatography and coulometric detection, *J.Chromatogr.B*, **1995**, 668, 291-297.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100  $\mu$ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50  $\mu$ L aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

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**HPLC VARIABLES**

**Column:** 300  $\times$  3.9 4  $\mu$ m NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic))  $\text{KH}_2\text{PO}_4$  adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

**Column temperature:** 30

**Flow rate:** 0.8

**Injection volume:** 50

**Detector:** UV 257

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**CHROMATOGRAM**

**Retention time:** 18.25

**Limit of detection:** <120 ng/mL

**KEY WORDS**

whole blood; plasma; interferences may occur—compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoyllecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melfalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyrindamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phenacyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

**REFERENCE**

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

**SAMPLE**

**Matrix:** blood, gastric contents, tissue, urine, vitreous humor

**Sample preparation:** Homogenize tissue with 4 volumes water. Extract 3 mL blood, gastric contents, urine, vitreous humor, or homogenized tissue with 1.5 mL saturated pH 9.5 ammonium chloride buffer and 5 mL chloroform:2-propanol:n-heptane 25:10:65 for 10 min. (Caution! Chloroform is a carcinogen!). Centrifuge at 3500 g for 10 min, evaporate the organic layer at 45°. Reconstitute with 30  $\mu$ L MeOH. Centrifuge at 10000 g for 5 min, remove 20  $\mu$ L of the supernatant, inject an aliquot.

**HPLC VARIABLES**

**Column:** 300  $\times$  3.9 4  $\mu$ m NovaPak C18

**Mobile phase:** MeOH:THF:10 mM pH 2.6 KH<sub>2</sub>PO<sub>4</sub> buffer 65:5:30

**Flow rate:** 0.8

**Injection volume:** 7

**Detector:** UV 228

**CHROMATOGRAM**

**Internal standard:** prochlorperazine

**OTHER SUBSTANCES**

**Extracted:** zuclopenthixol



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**KEY WORDS**

liver; kidney; lung; brain; skeletal muscle; prochlorperazine is IS

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**REFERENCE**

Tracqui,A.; Kintz,P.; Cirimele,V.; Berthault,F.; Mangin,P.; Ludes,B. HPLC-DAD and HPLC-MS findings in a fatality involving (Z)-cis-clopenthixol (zuclopenthixol), *J.Anal.Toxicol.*, **1997**, 21, 314–318.

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**SAMPLE**

**Matrix:** formulations

**Sample preparation:** Tablets. Pulverize tablet, add 1 L water, stir for 2 h, filter. Remove 9 mL filtrate, add 1 mL 100 µg/mL imipramine hydrochloride in water, mix, inject a 200 µL aliquot. Injections. Dilute a 2 mL aliquot to 1 L with water, mix. Remove a 9 mL aliquot, add 1 mL 100 µg/mL imipramine hydrochloride in water, mix, inject a 200 µL aliquot. Syrup. Dilute a 1 mL aliquot to 100 mL with water, mix. Remove a 9 mL aliquot, add 1 mL 100 µg/mL imipramine hydrochloride in water, mix, inject a 200 µL aliquot. Suppositories. Weigh out amount equivalent to about 1 mg prochlorperazine, add 80 mL pentane:isopropanol 97:3, shake thoroughly, stir for 15 min, make up to 100 mL with pentane:isopropanol 97:3, mix thoroughly, filter. Remove an aliquot, add imipramine, evaporate to dryness under a stream of nitrogen, reconstitute in 200 µL mobile phase, inject whole sample.

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**HPLC VARIABLES**

**Guard column:** Guard Pak CN

**Column:** µBondapak CN (RCM 8 × 10)

**Mobile phase:** MeCN:18 mM sodium acetate 95:5

**Flow rate:** 4

**Injection volume:** 200

**Detector:** UV 250

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**CHROMATOGRAM**

**Retention time:** 6

**Internal standard:** imipramine (4)

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**KEY WORDS**

injections; saline; 5% dextrose; tablets; syrup; suppositories; protect from light

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**REFERENCE**

el-Yazigi,A.; Wahab,F.A.; Afrane,B. Stability study and content uniformity of prochlorperazine in pharmaceutical preparations by liquid chromatography, *J.Chromatogr.A*, **1995**, 690, 71–76.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250 × 4 ODS (Hitachi)

**Mobile phase:** MeCN:50 mM phosphoric acid 45:55 containing 300 mM KCl

**Column temperature:** 55

**Flow rate:** 0.6

**Injection volume:** 20

**Detector:** UV 254

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**OTHER SUBSTANCES**

**Also analyzed:** albendazole, epirizole

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**REFERENCE**

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, 87, 960–966.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

#### HPLC VARIABLES

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

#### CHROMATOGRAM

**Retention time:** 4.5

#### OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclozine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylegonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlylcypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelethamine, triprolidine, tryptamine, verapamil, xylometazoline

#### REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, 323, 191–225.

#### SAMPLE

**Matrix:** solutions

**Sample preparation:** Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.

#### HPLC VARIABLES

**Column:** 300 × 3.9 10 µm µBondapak C18

**Mobile phase:** MeOH:acetic acid:triethylamine:water 70:1.5:0.5:28

**Flow rate:** 1.5

**Injection volume:** 10

**Detector:** UV 254

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## CHROMATOGRAM

**Retention time:** 11

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## OTHER SUBSTANCES

**Simultaneous:** mesoridazine, promethazine, acetophenazine, chlorpromazine, thioridazine, butaperazine, thiethylperazine

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## REFERENCE

Roos, R.W.; Lau-Cam, C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J. Chromatogr.*, **1986**, 370, 403–418.

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## SAMPLE

**Matrix:** solutions

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## HPLC VARIABLES

**Column:** 250 × 4.5 µm LiChrospher 100 RP-8

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 60:25:15 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

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## CHROMATOGRAM

**Retention time:** 8.18

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## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenopropfen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazinol, mefenamic acid, meperidine, mephentoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spirinolactone, sulfipyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimetoprim, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

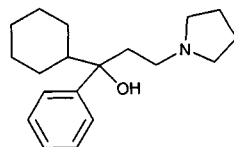
**KEY WORDS**

details of plasma extraction

**REFERENCE**

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J. Chromatogr. A*, **1995**, 692, 103–119.

# Procyclidine

**Molecular formula:** C<sub>19</sub>H<sub>29</sub>NO**Molecular weight:** 287.45**CAS Registry No.:** 77-37-2, 1508-76-5 (HCl)**Merck Index:** 7944**Lednicer No.:** 1 47**SAMPLE****Matrix:** solutions**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.**HPLC VARIABLES****Column:** 125 × 4.9 Spherisorb S5W silica**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7**Flow rate:** 2**Injection volume:** 20**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V**CHROMATOGRAM****Retention time:** 2.7**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinone, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenylglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperido-

late, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleppamine, triprolidine, tryptamine, verapamil, xylometazoline

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## REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, 323, 191–225.

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## SAMPLE

**Matrix:** solutions

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## HPLC VARIABLES

**Column:** 150 × 2 Deltabond C8 (Keystone)

**Mobile phase:** MeCN:2-butanone:50 mM pH 7.0 phosphate buffer 27:13:60

**Flow rate:** 0.15

**Injection volume:** 1

**Detector:** Chemiluminescence following post-column reaction. A 1 mM solution of Ru(2,2'-bipyridine)<sub>3</sub><sup>2+</sup> in 50 mM sodium sulfate (continuously sparged with helium) was oxidized to Ru(2,2'-bipyridine)<sub>3</sub><sup>3+</sup> using a Princeton Applied Research Model 174A polarographic analyzer with a platinum gauze working electrode, a platinum wire auxiliary electrode, and a silver wire reference electrode. The Ru solution at 0.3 mL/min was mixed with the column effluent in the flow cell of the detector, a fluorescence detector with the light source removed.

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## CHROMATOGRAM

**Retention time:** 5

**Limit of detection:** 0.1–1 µg/mL

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## OTHER SUBSTANCES

**Simultaneous:** dicyclomine

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## REFERENCE

Holeman, J. A.; Danielson, N. D. Microbore liquid chromatography of tertiary amine anticholinergic pharmaceuticals with tris(2,2'-bipyridine)ruthenium(III) chemiluminescence detection, *J. Chromatogr. Sci.*, **1995**, 33, 297–302.

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## SAMPLE

**Matrix:** solutions

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## HPLC VARIABLES

**Column:** 250 × 4 5 µm LiChrospher 100 RP-8

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 60:25:15 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

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## CHROMATOGRAM

**Retention time:** 4.65

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordi-azepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphen-oxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flur-azepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloper- idol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxy- chloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazin- dol, mefenamic acid, meperidine, mephénytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, met- ronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymet- azoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, phen- iramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltolox- amine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, promazine, promethazine, propafenone, propanthe- line, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, race- methorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, ser- traline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocin- ide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, trifluopromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

## KEY WORDS

details of plasma extraction

## REFERENCE

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, 692, 103-119.

# Progabide

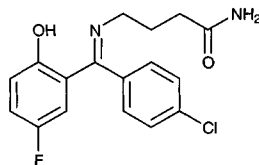
**Molecular formula:** C<sub>17</sub>H<sub>16</sub>ClFN<sub>2</sub>O<sub>2</sub>

**Molecular weight:** 334.78

**CAS Registry No.:** 62666-20-0

**Merck Index:** 7955

**Lednicer No.:** 4 47



## SAMPLE

**Matrix:** blood

**Sample preparation:** Evaporate 20 µL of a 20 µg/mL solution of IS in MeCN into the bottom of a tube using a stream of nitrogen, add 500 µL plasma, add 500 µL 2 M sodium acetate adjusted to pH 4.9 with HCl, add 9 mL hexane:isopropanol 96:4, shake for 5 min, centrifuge. Remove the organic layer and add it to a glass tube (pretreated with MeOH:triethylamine 80:20), add 500 µL 0.4% sodium borohydride in EtOH, vortex, let stand at room temperature for 10 min, add 2 mL 250 mM sodium citrate (adjusted to pH 2 with HCl), shake for 5 min, centrifuge, discard the organic layer, add 9 mL hexane:isopropanol, shake for 5 min, centrifuge, discard the organic layer. Add 200 µL 5 M NaOH to the aqueous layer, add 500 µL 1 M sodium citrate adjusted to pH 4.8 with HCl, add 9 mL dichloromethane, shake for 5 min, centrifuge.

Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 400  $\mu$ L MeOH, inject a 10  $\mu$ L aliquot.

#### HPLC VARIABLES

**Guard column:** 30  $\times$  4.6 5  $\mu$ m Spheri-5 RP-18

**Column:** 100  $\times$  9.4 5  $\mu$ m RAC Partisil 5 ODS-3

**Mobile phase:** MeOH:buffer 70:30 (Buffer was 33.3 mM  $\text{KH}_2\text{PO}_4$  adjusted to pH 5.06 with 33.3 mM  $\text{K}_2\text{HPO}_4$ .)

**Flow rate:** 2

**Injection volume:** 10

**Detector:** E, Bioanalytical Systems BAS LC-4A, TL-5 glassy carbon electrode 1 V

#### CHROMATOGRAM

**Retention time:** 4.5

**Internal standard:** 4-[[[(4-chlorophenyl)(5-chloro-2-hydroxyphenyl)methylene]amino]butanamide (SL 78050) (6.5)

**Limit of detection:** 30 ng/mL

#### OTHER SUBSTANCES

**Extracted:** metabolites

#### KEY WORDS

derivatization; pharmacokinetics; plasma

#### REFERENCE

Yonekawa, W.; Kupferberg, H.J.; Lambert, T. Measurement of progabide and its deaminated metabolite in plasma by high-performance liquid chromatography and electrochemical detection, *J. Chromatogr.*, **1983**, 276, 103–110.

#### SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Plasma + 70  $\mu$ L 10  $\mu$ g/mL thiopental in water + 1.2 mL buffer, mix gently, add 10 mL toluene, shake for 5 min (break any emulsion with sonication), centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 100  $\mu$ L MeOH, inject a 50  $\mu$ L aliquot. (Buffer was 100 mM acetic acid:100 mM sodium acetate 76:24, pH 4.5.)

#### HPLC VARIABLES

**Column:** 300  $\times$  4.6 5  $\mu$ m Nucleosil C18

**Mobile phase:** MeCN:MeOH:buffer:150 mM NaCl 27:27:36:10 (Buffer was 9.00 g  $\text{KH}_2\text{PO}_4$  and 140 mg  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$  in 1 L water, pH 5.05.)

**Flow rate:** 2.5

**Injection volume:** 20

**Detector:** UV 340

#### CHROMATOGRAM

**Retention time:** 7.9

**Internal standard:** thiopental (4)

**Limit of detection:** 20 ng/mL

#### KEY WORDS

plasma

#### REFERENCE

Decourt, J.P.; Mura, P.; Papet, Y.; Piriou, A.; Reiss, D. Simultaneous determination of progabide and its acid metabolite by reversed-phase high-performance liquid chromatography, *J. Chromatogr.*, **1990**, 527, 214–219.

#### SAMPLE

**Matrix:** blood, urine

**Sample preparation:** 1 mL Plasma, whole blood, urine + 50  $\mu$ L 10  $\mu$ g/mL IS in MeOH + 500  $\mu$ L 2 M pH 4.5 acetate buffer + 8 mL toluene, shake on a rotary shaker for 20 min, centrifuge

at 4° at 1000 g for 10 min. Remove the organic layer and add it to 500  $\mu\text{L}$  0.5% sodium borohydride in EtOH, vortex vigorously, let stand at room temperature for 20 min, add 2 mL 250 mM pH 1.8 citrate buffer, extract for 20 min. Remove the aqueous layer and add 200  $\mu\text{L}$  5 M NaOH, add 500  $\mu\text{L}$  1 M pH 7.7 citrate buffer, add 7 mL freshly distilled diethyl ether, extract for 20 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200  $\mu\text{L}$  MeOH:15 mM pH 7.1 phosphate buffer 40:60, inject a 100  $\mu\text{L}$  aliquot.

#### HPLC VARIABLES

**Column:** 150  $\times$  4.6 3  $\mu\text{m}$  Hypersil ODS

**Mobile phase:** MeCN:MeOH:33 mM pH 5.05 phosphate buffer:1.5 M NaCl 30:30:40:9

**Column temperature:** 54

**Flow rate:** 1

**Injection volume:** 100

**Detector:** E, Kipp Analytica Model 9205, +850 mV, Ag/AgCl reference electrode

#### CHROMATOGRAM

**Retention time:** 4.1

**Internal standard:** 4-[[[(4-chlorophenyl)(5-chloro-2-hydroxyphenyl)methylene]amino]butanamide (SL 78050) (5.9)

**Limit of detection:** 10 ng/mL

#### OTHER SUBSTANCES

**Extracted:** metabolites

**Noninterfering:** carbamazepine, carbamazepine epoxide, ethosuximide, phenobarbital, phenytoin, valproic acid

#### KEY WORDS

plasma; whole blood; derivatization

#### REFERENCE

Padovani,P.; Deves,C.; Bianchetti,G.; Thenot,J.P.; Morselli,P.L. Determination of progabide and its main acid metabolite in biological fluids using high-performance liquid chromatography and electrochemical detection. Application to the measurement of blood/plasma partition ratio, *J.Chromatogr.*, **1984**, 308, 229–239.

# Progesterone

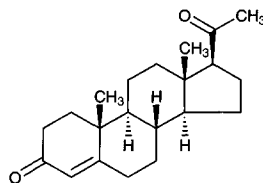
**Molecular formula:**  $\text{C}_{21}\text{H}_{30}\text{O}_2$

**Molecular weight:** 314.47

**CAS Registry No.:** 57-83-0

**Merck Index:** 7956

**Lednicer No.:** 2 164



#### SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 100  $\mu\text{L}$  2  $\mu\text{g/mL}$  dexamethasone in EtOH:water 10:90 + 100  $\mu\text{L}$  250 mM NaOH + 7 mL ether:dichloromethane 60:40, vortex for 30 s, centrifuge at 2000 rpm for 5 min. Remove 6 mL of the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 100  $\mu\text{L}$  dichloromethane:EtOH:water 95:4:1, inject a 50  $\mu\text{L}$  aliquot.

#### HPLC VARIABLES

**Column:** 250  $\times$  4.5 5  $\mu\text{m}$  Partisil silica

**Mobile phase:** Dichloromethane:EtOH:water 95:4:1

**Flow rate:** 1.5

**Injection volume:** 50

**Detector:** UV 239



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**CHROMATOGRAM****Retention time:** 2**Internal standard:** dexamethasone (11.5)**Limit of quantitation:** 25 ng/mL

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**OTHER SUBSTANCES****Extracted:** corticosterone, 11-deoxycortisol, hydrocortisone, 17-hydroxyprogesterone, 6 $\alpha$ -methylprednisolone, prednisolone, prednisone

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**KEY WORDS**plasma; normal phase

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**REFERENCE**Scott, N.R.; Chakraborty, J.; Marks, V. Determination of prednisolone, prednisone, and cortisol in human plasma by high-performance liquid chromatography, *Anal. Biochem.*, **1980**, 108, 266–268.

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**SAMPLE****Matrix:** blood**Sample preparation:** Prepare an SPE column by suspending 250 mg 80-120 mesh Carbo-pack B (Supelco) in chloroform and adding to a 150  $\times$  6 glass column, wash with 5 mL MeOH, wash with 10 mL water. 1 mL Serum + 9 mL MeOH:water 35:65, add to the SPE column, rinse vial with two 5 mL portions of water, add rinses to SPE column, wash with 15 mL MeCN, wash with 1 mL MeCN:chloroform 70:30, elute with 5 mL MeOH:chloroform 10:90. Collect 4.5 mL eluate (as soon as eluant is added to column), evaporate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 50  $\mu$ L MeCN:MeOH 50:50, inject a 20  $\mu$ L aliquot.

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**HPLC VARIABLES****Guard column:** 5  $\mu$ m C18 (PoliConsult Scientifica, Rome)**Column:** 250  $\times$  4.6 5  $\mu$ m C18 (PoliConsult Scientifica, Rome)**Mobile phase:** MeCN:water 46:54**Flow rate:** 1.6**Injection volume:** 20**Detector:** UV 242

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**CHROMATOGRAM****Retention time:** 13.6**Limit of detection:** 0.2 ng/mL

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**OTHER SUBSTANCES****Simultaneous:** adrenosterone, aldosterone, androstenedione, corticosterone, cortisone, 11-dehydrocorticosterone, 11-deoxycorticosterone, 11-deoxycortisol, 21-deoxycortisol, 20 $\alpha$ -dihydroprogesterone, hydrocortisone, 19-hydroxyandrostenedione, 18-hydroxycorticosterone, 6 $\alpha$ -hydroxyprogesterone, 16 $\alpha$ -hydroxyprogesterone, 17 $\alpha$ -hydroxyprogesterone, 7 $\alpha$ -hydroxytestosterone, prednisolone, prednisone, testosterone**Noninterfering:** acebutolol, allopurinol, aminophylline, amitriptyline, aspirin, caffeine, carbamazepine, diazepam, digoxin, disopyramide, doxepin, indapamide, meprobamate, methyl dopa, oxazepam, phenobarbital, propranolol, theophylline, ascorbic acid

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**KEY WORDS**serum; SPE

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**REFERENCE**Laganà, A.; D'Ascenzo, G.; Marino, A.; Tarola, A.M. Liquid-chromatographic determination of progesterone in serum, with spectrophotometric detection, *Clin. Chem.*, **1986**, 32, 508–510.

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**SAMPLE****Matrix:** blood**Sample preparation:** Condition a Bond-Elut C18 SPE cartridge with MeOH and water. Add 500  $\mu$ L plasma to the SPE cartridge, wash with 2 mL water, wash with 2 mL MeOH:water 20:80, elute with two 500  $\mu$ L aliquots of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50  $\mu$ L MeOH:water 20:80, inject a 20  $\mu$ L aliquot.

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**HPLC VARIABLES****Column:** 100 × 1.5 µm Hypersil ODS**Mobile phase:** MeCN:MeOH:water 25:25:50**Flow rate:** 0.1**Injection volume:** 20**Detector:** UV (wavelength not given)

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**CHROMATOGRAM****Retention time:** 17**Limit of detection:** 2 ng/mL

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**OTHER SUBSTANCES****Extracted:** androstenedione, 20α-hydroxy-4-pregnen-3-one, 17α-hydroxyprogesterone, norethindrone, testosterone

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**KEY WORDS**

microbore; rat; plasma; SPE

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**REFERENCE**Taylor,R.B.; Kendle,K.E.; Reid,R.G.; Hung,C.T. Chromatography of progesterone and its major metabolites in rat plasma using microbore high-performance liquid chromatography columns with conventional injection and detection systems, *J.Chromatogr.*, **1987**, 385, 383–392.

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**SAMPLE****Matrix:** blood**Sample preparation:** 100 µL Serum + 500 µL water + 100 µL 10 µg/mL 3,7-dimethoxyflavone in EtOH + 8 mL diethyl ether, shake, centrifuge at 4° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 100 µL MeOH:water 40:60, inject a 50 µL aliquot.

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**HPLC VARIABLES****Column:** 250 × 4.6 3 µm NS-Gel C18**Mobile phase:** Gradient. MeOH:water from 40:60 to 55:45, maintain at 55:45 for 24 min, to 80:20 over 25 min**Column temperature:** 50**Flow rate:** 1**Injection volume:** 50**Detector:** UV 210, UV 240

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**CHROMATOGRAM****Retention time:** 52.25**Internal standard:** 3,7-dimethoxyflavone (47)

---

**OTHER SUBSTANCES****Extracted:** aldosterone, androstenedione, dehydroepiandrosterone, deoxycorticosterone, 11-deoxycortisol, estradiol, estrone, hydrocortisone, 17-hydroxyprogesterone, pregnenolone

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**KEY WORDS**

serum

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**REFERENCE**Ueshiba,H.; Segawa,M.; Hayashi,T.; Miyachi,Y.; Irie,M. Serum profiles of steroid hormones in patients with Cushing's syndrome determined by a new HPLC/RIA method, *Clin.Chem.*, **1991**, 37, 1329–1333.

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**SAMPLE****Matrix:** blood**Sample preparation:** Extract 1 mL serum twice with 5 volumes ether by vortexing for 2 min, evaporate extracts to dryness under a stream of nitrogen at 35°, reconstitute in 100 µL MeOH.

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**HPLC VARIABLES**

**Column:** 240 × 4.5 Bio-Rad ODS-5S

**Mobile phase:** Gradient. MeOH:MeCN:water at 20:60:20 for 3 min then to 5:85:10 over 26 min

**Flow rate:** 1

**Injection volume:** 50

**Detector:** UV 230

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**OTHER SUBSTANCES**

**Simultaneous:** estradiol, androstenedione, testosterone

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**KEY WORDS**

serum

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**REFERENCE**

Yu, F.H.; Yun, Y.W.; Yuen, B.H.; Moon, Y.S. Effects of hydroxyflutamide on rats treated with a superovulatory dose of pregnant mare serum gonadotropin, *Can. J. Physiol. Pharmacol.*, **1991**, 69, 185–190.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 1 mL Serum + 100 µL water containing 5 µg/mL 2,3-diaminonaphthalene and 3.5 µg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30–40°, reconstitute the residue in 70 µL MeOH:100 mM perchloric acid 50:50, inject a 20 µL aliquot.

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**HPLC VARIABLES**

**Column:** 150 × 3.9 4 µm Nova-Pak C18

**Mobile phase:** Gradient. A was 58 mM NaH<sub>2</sub>PO<sub>4</sub> containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 245, 256, 343

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**CHROMATOGRAM**

**Retention time:** 25.66

**Internal standard:** 2,3-diaminonaphthalene (10.71), 18-hydroxy-11-deoxycorticosterone (15.85)

**Limit of detection:** 1–10 ng/mL (245 nm)

---

**OTHER SUBSTANCES**

**Extracted:** betamethasone, chloroquine, corticosterone, cortisone, dexamethasone, fluendrenolide, fluocinolone acetonide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, 17β-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, prednisone, triamcinolone

**Noninterfering:** aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

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**KEY WORDS**

serum

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**REFERENCE**

Volin, P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids. *J. Chromatogr. B*, **1995**, 666, 347–353.

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**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation.)

Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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**HPLC VARIABLES**

**Guard column:** 20 mm long Symmetry C18

**Column:** 250 × 4.6 5 µm Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 242.9

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**CHROMATOGRAM**

**Retention time:** 23.835

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**KEY WORDS**

whole blood

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**REFERENCE**

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, 763, 149-163.

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**SAMPLE**

**Matrix:** formulations

**Sample preparation:** Dilute 0.5 mL of nanocapsules suspension 1:200 with MeCN, filter, inject an aliquot. Alternatively, evaporate 5 mL of a nanocapsule suspension to dryness and dissolve the residue in 150 mL dichloromethane or ethyl acetate, dry over anhydrous sodium sulfate. Evaporate to dryness under reduced pressure, take up the residue in 50 mL MeOH, dilute 1:20 with MeOH, inject an aliquot.

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**HPLC VARIABLES**

**Column:** 250 × 4 5 µm Nucleosil C18

**Mobile phase:** MeCN: water 75:25

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 6.01

**Limit of detection:** 500 ng/ml

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**OTHER SUBSTANCES**

**Simultaneous:** benzyl benzoate

**Noninterfering:** poly-ε-caprolactone

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**KEY WORDS**

nanocapsules

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**REFERENCE**

Benali,S.; Tharasse-Bloch,C.; André, D; Vérité, P.; Duclos,R.; Lafont,O. Determination of progesterone in nanocapsules by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, 20, 3233-3243.

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**SAMPLE**

**Matrix:** formulations

**Sample preparation:** Injections. Extract 2 mL with EtOH:water 85:15, make up extracts to 100 mL with EtOH:water 85:15, remove a 2 mL aliquot and add it to 1 mL 1 mg/mL hydrocortisone in EtOH. Dilute this mixture to 50 mL with EtOH:water 50:50, inject an aliquot. Suspensions.

Dilute 2 mL suspension to 100 mL with EtOH, filter (if necessary), remove a 2 mL aliquot and add it to 1 mL 1 mg/mL hydrocortisone in EtOH. Dilute this mixture to 50 mL with EtOH, inject an aliquot.

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**HPLC VARIABLES**

**Column:** 300 × 4  $\mu$ Bondapak CN

**Mobile phase:** MeOH:20 mM  $\text{KH}_2\text{PO}_4$  30:70

**Flow rate:** 2

**Injection volume:** 20

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 6.5

**Internal standard:** hydrocortisone (2.5)

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**OTHER SUBSTANCES**

**Simultaneous:** medroxyprogesterone acetate

**Noninterfering:** polyethylene glycol 4000, myristyl-gamma-picolinium chloride, methylcellulose, thimerosal

**Interfering:** benzyl benzoate

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**REFERENCE**

Das Gupta, V. Quantitation of hydroxyprogesterone caproate, medroxyprogesterone acetate, and progesterone by reversed-phase high-pressure liquid chromatography, *J.Pharm.Sci.*, **1982**, 71, 294–297.

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**SAMPLE**

**Matrix:** formulations

**Sample preparation:** Grind tablets, weigh out amount equivalent to 0.1 mg digitoxin, add 500  $\mu$ L MeOH:water 50:50, sonicate for 5 min, add 5 mL 12.6  $\mu$ g/mL progesterone in acetone:EtOH 90:10, sonicate for 10 min, centrifuge at 1400 g for 5 min. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute the residue in 100  $\mu$ L MeOH, inject a 0.5  $\mu$ L aliquot.

---

**HPLC VARIABLES**

**Column:** 102 × 0.5 5  $\mu$ m SC-01 ODS in a PTFE tube (Japan Spectroscopic)

**Mobile phase:** MeCN:MeOH:water 10:20:17

**Flow rate:** 0.008

**Injection volume:** 0.5

**Detector:** UV 220

---

**CHROMATOGRAM**

**Retention time:** 23

**Internal standard:** progesterone

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**OTHER SUBSTANCES**

**Simultaneous:** digitoxigenin, digitoxigenin monodigitoxoside, digitoxigenin bisdigitoxoside, digitoxin

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**KEY WORDS**

microbore; tablets; progesterone is IS

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**REFERENCE**

Fujii, Y.; Ikeda, Y.; Yamazaki, M. Determination of cardiac glycosides in digitoxin tablets and deslanoside injections by micro-HPLC, *J.Chromatogr.Sci.*, **1990**, 28, 288–291.

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**SAMPLE**

**Matrix:** formulations

**Sample preparation:** Place a 100 mg capsule in 1 mL water, heat to dissolve, add 2 mL 2 mg/mL progesterone in MeOH, make up to 100 mL with MeOH, allow to settle, filter (0.45  $\mu$ m), inject a 20  $\mu$ L aliquot of the filtrate.

---

**HPLC VARIABLES**

**Column:** 300 × 4.6 10 µm 100 Å Chromegabond C-22 (ES Industries)

**Mobile phase:** MeCN:water 70:30 containing 5 mM tetrabutylammonium phosphate (Pic A)

**Flow rate:** 1.8

**Injection volume:** 20

**Detector:** UV 214

---

**CHROMATOGRAM**

**Retention time:** 6.8

**Internal standard:** progesterone

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**OTHER SUBSTANCES**

**Simultaneous:** docusate

**Noninterfering:** ferrous fumarate, casanthranol

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**KEY WORDS**

capsules; progesterone is IS

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**REFERENCE**

Hogue,D.R.; Zimmardi,J.A.; Shah,K.A. High-performance liquid chromatographic analysis of docusate sodium in soft gelatin capsules, *J.Pharm.Sci.*, **1992**, *81*, 359–361.

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**SAMPLE**

**Matrix:** formulations

**Sample preparation:** Sonicate 100 mg hydrogel in 50 mL MeOH for 15 min, cool to room temperature, make up to 100 mL with MeOH, filter (0.45 µm), inject a 20 µL aliquot.

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**HPLC VARIABLES**

**Column:** 250 × 5 5 µm Nucleosil C18

**Mobile phase:** MeOH:water 90:10

**Column temperature:** 40

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 240

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**CHROMATOGRAM**

**Retention time:** 3.9

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**KEY WORDS**

hydrogels

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**REFERENCE**

Valenta,C.; Schmatzberger-Wagerer,M. Stabilitätsuntersuchungen von Progesteron-Hydrogelen [Stability testing of progesterone hydrogels], *Pharmazie*, **1995**, *50*, 69–70.

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**SAMPLE**

**Matrix:** microsomal incubations

**Sample preparation:** 500 µL Microsomal incubation + 500 µL MeCN, centrifuge, inject a 50 µL aliquot of the supernatant.

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**HPLC VARIABLES**

**Guard column:** 45 mm long 5 µm Ultrasphere C18

**Column:** 150 mm long 5 µm Ultrasphere C8

**Mobile phase:** MeCN:MeOH:water 27.5:27.5:45

**Flow rate:** 1.5

**Injection volume:** 50

**Detector:** UV 240

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**CHROMATOGRAM**

**Retention time:** 22.6

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**OTHER SUBSTANCES**

**Extracted:** deoxycorticosterone

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**KEY WORDS**

rat; liver

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**REFERENCE**

Cribb,A.E.; Spielberg,S.P.; Griffin,G.P. N<sub>1</sub>-Hydroxylation of sulfamethoxazole by cytochrome P450 of the cytochrome P4502C subfamily and reduction of sulfamethoxazole hydroxylamine in human and rat hepatic microsomes, *Drug Metab.Dispos.*, **1995**, 23, 406–414.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 200 × 4.6 5 μm Hypersil ODS

**Mobile phase:** MeCN:water 60:40

**Column temperature:** 37

**Flow rate:** 1.5

**Detector:** UV 226

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**CHROMATOGRAM**

**Retention time:** 4.51

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**OTHER SUBSTANCES**

**Simultaneous:** estradiol

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**REFERENCE**

Kim,D.-D.; Kim,J.L.; Chien,Y.W. Mutual hairless rat skin permeation-enhancing effect of ethanol/water system and oleic acid, *J.Pharm.Sci.*, **1996**, 85, 1191–1195.

---

**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

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**HPLC VARIABLES**

**Column:** 300 × 3.9 10 μm μBondapak C18

**Mobile phase:** MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38

**Flow rate:** 1.5

**Injection volume:** 10

**Detector:** UV 240

---

**CHROMATOGRAM**

**Retention time:** 28

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**OTHER SUBSTANCES**

**Simultaneous:** prednisone, prednisolone, prednisolone succinate, hydrocortisone acetate, norethindrone, methyltestosterone

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**REFERENCE**

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, 370, 403–418.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Inject an aliquot of a solution in MeOH.

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**HPLC VARIABLES**

**Column:** Radial-PAK μBondapak C18

**Mobile phase:** MeCN:water 50:50

**Flow rate:** 2

**Injection volume:** 100

**Detector:** UV 254 or 214

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**CHROMATOGRAM**

**Retention time:** 16.3

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**OTHER SUBSTANCES**

**Simultaneous:** estrone, estriol, estradiol, testosterone

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**REFERENCE**

Erkoc,F.U.; Özsar,S.; Güven,B.; Kalkandelen,G.; Ugrar,E. High-performance liquid chromatographic analysis of steroid hormones, *J.Chromatogr.Sci.*, **1989**, 27, 86–90.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 150 × 6 5 µm Shim-pack CLC-ODS

**Mobile phase:** MeOH:THF:water 26:18:56

**Column temperature:** 48

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 240

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**CHROMATOGRAM**

**Retention time:** 23.4

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**OTHER SUBSTANCES**

**Simultaneous:** cortisone, estriol, cortisol, corticosterone, 11-deoxycortisol, androstenedione, prednisone acetate, 11-deoxycorticosterone, testosterone, 17α-hydroxyprogesterone, dexamethasone acetate, estradiol, estrone

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**REFERENCE**

Wei,J.Q.; Wei,J.L.; Zhou,X.T. Optimization of an isocratic reversed phase liquid chromatographic system for the separation of fourteen steroids using factorial design and computer simulation, *Biomed.Chromatogr.*, **1990**, 4, 34–38.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 0.5 mg/mL solution in MeOH, inject a 5 µL aliquot.

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**HPLC VARIABLES**

**Column:** 250 × 4.6 Zorbax RX

**Mobile phase:** Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN: water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.

**Column temperature:** 30

**Flow rate:** 2

**Injection volume:** 5

**Detector:** UV 210

---

**CHROMATOGRAM**

**Retention time:** 27.6

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**OTHER SUBSTANCES**

**Simultaneous:** acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranlylcypromine, tripeleennamine



**KEY WORDS**

details for purification of triethylamine in paper

**REFERENCE**

Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, 16, 3941-3964.

**SAMPLE**

**Matrix:** solutions

**HPLC VARIABLES**

**Column:** 250 × 4.6 Zorbax RX

**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

**Column temperature:** 30

**Flow rate:** 2

**Detector:** UV 210

**OTHER SUBSTANCES**

**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitrityline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlor-diazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazalamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methypylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscaphine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrrhyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, transcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

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**REFERENCE**

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 25 µg/mL solution in mobile phase, inject an aliquot.

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**HPLC VARIABLES**

**Column:** 250 × 4.6 Partisil 10 ODS-1

**Mobile phase:** MeOH:water 55:45

**Column temperature:** 40

**Flow rate:** 1.5

**Detector:** UV 240

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**CHROMATOGRAM**

**Retention time:** k' 7.649

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**OTHER SUBSTANCES**

**Also analyzed:** androsterone (UV 210), cortexolone (UV 240), cortisone (UV 240), estradiol (UV 280), estrone (UV 280), ethinyl estradiol (UV 280), ethisterone (UV 240), hydrocortisone (UV 240), hydroxyprogesterone (UV 240), lynestrenol (UV 210), medroxyprogesterone acetate (UV 240), medroxyprogesterone (UV 240), methandienone (UV 240), methylandrostenediol (UV 210), methylprednisolone acetate (UV 240), methylprednisolone (UV 240), methyltestosterone (UV 240), nandrolone (UV 240), norethisterone (UV 240), prednisolone acetate (UV 240), prednisolone (UV 240), prednisone (UV 240), pregnenolone (UV 210), testosterone (UV 240)

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**REFERENCE**

Sadlej-Sosnowska,N. Structure retention relationship for steroid hormones. Functional groups as structural descriptors, *J.Liq.Chromatogr.*, **1994**, *17*, 2319–2330.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a solution in MeCN:water 60:40 containing 0.3% Tween 80, inject a 50 µL aliquot.

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**HPLC VARIABLES**

**Column:** 300 × 3.9 Novapak C18

**Mobile phase:** MeCN:water 60:40

**Flow rate:** 2

**Injection volume:** 50

**Detector:** UV 248

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**OTHER SUBSTANCES**

**Simultaneous:** levonorgestrel

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**REFERENCE**

Gao,Z.-H.; Shukla,A.J.; Johnson,J.R.; Crowley,W.R. Controlled release of a contraceptive steroid from biodegradable and injectable gel formulations: In vitro evaluation, *Pharm.Res.*, **1995**, *12*, 857–863.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Inject a 20 µL aliquot of a solution in MeOH:water 50:50.

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**HPLC VARIABLES**

**Column:** 250 × 4 7 µm LichroCART RP-8 (Merck)

**Mobile phase:** MeCN:MeOH:water 32:37:31

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 230

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**CHROMATOGRAM****Retention time:** 9

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**OTHER SUBSTANCES****Simultaneous:** fluoxymesterone, medrogestone, mestranol, norethindrone, testosterone propionate

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**REFERENCE**

Gau,Y.S.; Sun,S.W.; Chem,R.R.-L. Optimization of high-performance liquid chromatographic separation for progestogenic, estrogenic, and androgenic steroids using factorial design, *J.Liq.Chromatogr.*, **1995**, *18*, 2373-2382.

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**SAMPLE****Matrix:** solutions

**Sample preparation:** 100  $\mu$ L 200  $\mu$ g/mL progesterone in toluene + 100  $\mu$ L 2.5% trifluoroacetic acid (?) in toluene + 100  $\mu$ L 1 mg/mL acenaphthene-5-sulfonyl hydrazine in EtOH:toluene 10:90, evaporate to dryness under reduced pressure at 60°, reconstitute with 100  $\mu$ L MeCN, inject a 20  $\mu$ L aliquot. (Preparation of acenaphthene-5-sulfonyl hydrazine is as follows. Dissolve 20 g acenaphthene in 100 g nitrobenzene, cool to 0°, add 9 mL chlorosulfonic acid dropwise with stirring, maintain the temperature below 5°, when the addition is complete allow the temperature to rise to 20° over 30 min, add 500 mL water. Remove the aqueous layer and neutralize it with solid sodium carbonate, heat and add NaCl until precipitation occurs, cool in an ice bath for 1 h, filter, heat at 140° to remove traces of water and nitrobenzene to give acenaphthene-5-sulfonic acid sodium salt as a pale yellow solid (mp >300°). Grind 10 g acenaphthene-5-sulfonic acid sodium salt with 3.5 g phosphorus pentachloride in a mortar for 3 min, add ice and water, extract with 100 mL ethyl acetate. Wash the ethyl acetate layer with 5% sodium bicarbonate and with water until neutral, dry over anhydrous sodium sulfate, evaporate the ethyl acetate under a stream of nitrogen, chromatograph on a 300  $\times$  20 column of silica gel H with toluene to give acenaphthene-5-sulfonyl chloride (mp 98-101°) as the first yellow band to elute. Cool a solution of 1 g acenaphthene-5-sulfonyl chloride in 3 mL THF to 10° and pass nitrogen through the solution, add 400  $\mu$ L 85% hydrazine hydrate dropwise with stirring, maintain the temperature between 10° and 15°, stir for a further 15 min. Filter the upper THF layer through Celite, wash the Celite with 1 mL THF. Stir the filtrate vigorously and add two 10 mL portions of water, cool in a refrigerator for 1 h, filter the precipitate, wash with water, dry, recrystallize from EtOH to give acenaphthene-5-sulfonyl hydrazine (mp 132-4°).)

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**HPLC VARIABLES****Column:** 250  $\times$  4.6 5  $\mu$ m Hypersil C18**Mobile phase:** Gradient. A was 0.5 g/L pH 7 Tris buffer. B was MeCN:water 90:10. A:B from 50:50 to 30:70 over 10 min.**Flow rate:** 2**Injection volume:** 20**Detector:** F ex 230 em 350

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**KEY WORDS**

derivatization

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**REFERENCE**

Gifford,L.A.; Owusu-Daaku,F.T.K.; Stevens,A.J. Acenaphthene fluorescence derivatization reagents for use in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *715*, 201-212.

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**SAMPLE****Matrix:** solutions**Sample preparation:** Inject a 10  $\mu$ L aliquot of a 100 ppm solution.

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**HPLC VARIABLES****Column:** 150  $\times$  4.6 Develosil ODS-5**Mobile phase:** Gradient. MeOH:water from 50:50 to 90:10 over 15 min**Flow rate:** 1**Injection volume:** 10

**Detector:** MS, JEOL JMS-SX102A reversed geometry (BE), accelerating voltage +5 kV, air pressure chemical ionization APCI, nebulizer 290°, ion source chamber 400°, discharge electrode, skimmer 1 aperture 300  $\mu\text{m}$ , skimmer 2 aperture 400  $\mu\text{m}$ , no nebulizer gas

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## CHROMATOGRAM

**Retention time:** 13

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## OTHER SUBSTANCES

**Simultaneous:** corticosterone, cortisone, hydrocortisone

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## REFERENCE

Nojima,K.; Fujimaki,S.; Hertsens,R.C.; Morita,T. Application of liquid chromatography-atmospheric pressure chemical ionization mass spectrometry to a sector mass spectrometer, *J.Chromatogr.A*, **1995**, 712, 17–19.

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## SAMPLE

**Matrix:** tissue

**Sample preparation:** Dry pack  $60 \times 8$  mm glass columns with 250 mg Carbowpack B (200-400 mesh) and  $60 \times 4$  mm glass columns with 50 mg Amberlite CG-400 I (100-200 mesh). Wash Carbowpack column with 5 mL MeOH, 15 mL dichloromethane:MeOH 70:30, and MeOH:water 85:15. Wash Amberlite column with 3 mL 0.5 M NaOH, 8 mL dichloromethane:MeOH 70:30, 1 mL water, and 3 mL 1 M HCl. Repeat this cycle 4 times. Finally pass through 20 mL 50 mM NaOH then 1 mL water. Keep column in water. (Process converts Amberlite to OH form.) Homogenize 1 g of tissue in 5 mL MeOH, sonicate 5 min, centrifuge at 6000 rpm for 10 min. Add another 5 mL MeOH to pellet and repeat. Combine supernatants, make up to 6.8 mL with MeOH, add 1.2 mL water. Pass through Carbowpack column, wash column with 2 mL MeOH:water 85:15 then 2 mL MeOH, elute column with 8 mL dichloromethane:MeOH 70:30. Pass eluate onto Amberlite column, add 1 mL MeOH to column, collect all eluates from column, evaporate to dryness under nitrogen at 40°, take up in 200  $\mu\text{L}$  MeOH:water 50:50, add 25  $\mu\text{L}$  10  $\mu\text{g/mL}$  p-chlorophenol, inject 50  $\mu\text{L}$  aliquot.

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## HPLC VARIABLES

**Guard column:**  $20 \times 4.6$  5  $\mu\text{m}$  Supelguard LC-18

**Column:**  $250 \times 4.6$  5  $\mu\text{m}$  Supelco C18

**Mobile phase:** Gradient. MeCN:water from 40:60 to 65:35 in 30 min

**Flow rate:** 1.2

**Injection volume:** 50

**Detector:** UV 242

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## CHROMATOGRAM

**Retention time:** 26

**Internal standard:** p-chlorophenol (7)

**Limit of detection:** 1 ng/g

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## OTHER SUBSTANCES

**Simultaneous:** testosterone, trenbolone

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## KEY WORDS

muscle; liver; chicken; ox

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## REFERENCE

Laganà,A.; Marino,A. General and selective isolation procedure for high-performance liquid chromatographic determination of anabolic steroids in tissues, *J.Chromatogr.*, **1991**, 588, 89–98.

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## SAMPLE

**Matrix:** urine

**Sample preparation:** Add 10 mL urine to a Supelclean LC-18 SPE tube at a flow rate of 2 mL/min, wash with 4 mL 25 mM sodium borate buffer, wash with 4 mL 40% MeOH, wash with 4 mL 20% acetone, elute with two 500  $\mu\text{L}$  aliquots of 73% MeOH, evaporate under nitrogen at 40°, reconstitute with 1 mL mobile phase, inject a 200  $\mu\text{L}$  aliquot.

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## HPLC VARIABLES

**Column:**  $250 \times 4.6$  5  $\mu\text{m}$  Microsorb silica

**Mobile phase:** Cyclohexane:ethyl acetate 40:60

**Injection volume:** 200

**Detector:** F ex 247 em 547, after post-column reaction with 30 mM Tb(NO<sub>3</sub>)<sub>3</sub> in ethyl acetate using a 50 cm tightly coiled capillary tube to ensure mixing

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#### CHROMATOGRAM

**Retention time:** 12

**Limit of detection:** 80 pg/mL

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#### OTHER SUBSTANCES

**Extracted:** testosterone acetate, bolasterone, testosterone

**Simultaneous:** 17-methyltestosterone

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#### KEY WORDS

SPE; normal phase; post-column reaction

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#### REFERENCE

Amin,M.; Harrington,K.; von Wandruszka,R. Determination of steroids in urine by micellar HPLC with detection by sensitized terbium fluorescence, *Anal.Chem.*, **1993**, 65, 2346–2351.

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## Proheptazine

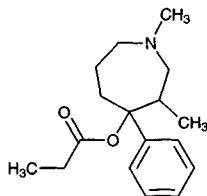
**Molecular formula:** C<sub>17</sub>H<sub>25</sub>NO<sub>2</sub>

**Molecular weight:** 275.39

**CAS Registry No.:** 77-14-5

**Merck Index:** 7959

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#### SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

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#### HPLC VARIABLES

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

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#### CHROMATOGRAM

**Retention time:** 3.9

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#### OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl,